EXHIBIT B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Michael Wayne Graham et al. Serial No.: 09/100,812 Filed: 19 June 1998 For: Synthetic genes and genetic constructs comprising same I	Examiner: Sumesh Kaushal/Scott Priebe Art Unit: 1633 DECLARATION OF: Ken C Reed

Commissioner of Patents and Trademarks
Washington D.C. 20231

Sir,

I, Kenneth Clifford Reed, state as follows:

My present position is Research Director of Benitec Australia Ltd, one of the assignees of the subject application (serial no. 09/100,812). I am authorised to make this declaration on behalf of the applicants.

I have read the above-captioned application and followed the prosecution thereof.

In my opinion, it is extremely advantageous to determine the function of a gene by delaying, repressing or reducing its expression in a whole, living animal. By so doing, the function of the gene is deduced from the effect of its reduced or absent expression. This gives more complete and useful information than delaying, repressing or reducing its expression *in vitro* in cell, tissue or organ culture since many genes have substantial secondary effects beyond those attributable to its immediate product. This is particularly so for hormones, growth factors, receptors, signal transduction factors, transcription factors, and the like.

A specific commercial motivation for determining gene function by delaying, repressing or reducing its expression in whole animals is in the validation of drug targets, whereby the potential target is delayed, repressed or reduced by reducing expression of the gene encoding it. This provides the method of choice for determining the effects to be expected of a potential drug that reduces the activity of a gene product.

In particular, the preferred and most studied model for human therapeutics is the rat in which gene knockout technology has not been successful. Accordingly, target validation *in vivo* has been carried out in gene knockout mice, which has resulted in

less accurate and less useful results. The ability to delay, repress or reduce expression of a gene in a rat *in vivo* was a well recognised and desired objective since before March 1998. The methods and genetic constructs of this invention meet this need.

Available methods for delaying, repressing or reducing the expression of a target gene are restricted to either random insertional mutagenesis or targeted gene knockout in mouse embryonic stem cells followed by reconstitution of chimeric embryos and breeding selection. The latter method has greater utility but is very slow, extremely expensive and restricted in its application to (a few strains of) inbred mice. The consequence is that many drug targets are not validated before the drug candidate enters clinical trials in humans, resulting in an increasing level of failure of drugs in early human trials.

I attach as Annexure KCR1 five pages from the website of Deltagen, Inc, a corporation which has commercialised mouse gene knockout technology of the type described above. These pages contain a one page description of the technology, followed by two press releases describing two separate collaborations between Stanford University and Merck & Co, Inc to use the gene knockout technology (of the type described above) for the purposes of studying gene function and validating drug targets. These are only two of many examples of work being conducted around the world utilising *in vivo* mouse models where a gene is not expressed or is down-regulated. As explained above, in my view, this gene knockout technology has significant limitations, but the need for such *in vivo* models is such that pre-eminent researchers are prepared to use these models despite their limitations.

I now describe how a scientist with routine skill and familiar with standard methods of molecular biology and biochemistry would have been able to repress, delay or otherwise reduce expression of a target gene in an animal *in vivo* from the teaching of the specification.

Co-suppression of tyrosinase in Mus musculus strains C57BL/6 and C57BL/6 x DB1 hybrid in vivo

1. Preparation of constructs

The interim plasmid TOPO.TYR and test plasmid pCMV.TYR.BGlZ.RYT were generated as described in the Declaration of Michael Graham made April 23, 2002 and made of record for this application.

2. *Generation of transgenic mice*

Transgenic mice were generated through genetic modification of pronuclei of zygotes. After isolation from oviducts, zygotes were placed on an injection microscope and the transgene, in the form of a purified DNA solution, was injected into the most visible pronucleus (U.S. Patent No. 4,873,191).

Pseudo-pregnant female mice were generated, to act as "recipient mothers", by induction into a hormonal stage that mimics pregnancy. Injected zygotes were then either cultured overnight in order to assess their viability, or transferred immediately back into the oviducts of pseudo-pregnant recipients. Of 421 injected zygotes, 255 were transferred. Transgenic off-spring resulting from these injections are called "founders". To determine that the transgene has integrated into the mouse genome, off-spring were genotyped after weaning. Genotyping was carried out by PCR and/or by Southern blot analysis on genomic DNA purified from a tail biopsy.

Founders were then mated to begin establishing transgenic lines. Founders and their offspring are maintained as separate pedigrees, since each pedigree varies in transgene copy number and/or chromosomal location. Therefore, each transgenic mouse generated by pronuclear injection was the founder of a new strain. Where the founder was female, some pups from the first litter were analyzed for transgene transmission.

Annexures KCR3 and KCR4 illustrate the selection of a transformed mouse. Annexure KCR3 is a Southern blot of Tyr(hp) transgenic founders. DNA was extracted from tail tip samples of mice born from zygotes injected with a tyrosinase hairpin construct (Tyr(hp)) into the male pronucleus, as described. Samples were digested with BamHI and probed with the CMV promoter. Mouse #75-038 was identified as transgenic (female). Annexure KCR4 is a Dot blot of A-generation progeny of Tyr(hp) transgenic founder #75-038. DNA was extracted from tail tip samples of A-generation progeny A036 and A037, as shown. Dot blot samples were probed with the CMV promoter or an endogenous control sequence (Shiraz 3'), as shown. A-generation mice were bred from mating #75-038 with a C57Bl/6 male. Mouse #75-A037 was identified as transgenic (female).

3. *Detection of co-suppression phenotype*

Visual read-out of successful transgenic mice is an alteration to coat colour. Skin-cell biopsies are harvested from transgenic mice and cultured as primary cultures of

melanocytes by standard methods (Bennett *et al.*, 1989; Spanakis *et al.*, 1992; Sviderskaya *et al.*, 1995).

Melanin pigmentation of transgenic A-generation mouse #75-A037 (from founder 038) was visually inspected (see Annexure KCR9) and found to be deficient in broad latero-dorsal areas and in the flanks, resulting from localised down-regulation of tyrosinase.

Co-suppression of α-1,3,-galactosyl transferase (GalT) in Mus musculus strain C57BL/6 in vivo

(a) Plasmid TOPO.GALT

Total RNA was purified from cultured murine 2.3D17 neural cells and cDNA prepared. To amplify the 3'-UTR of the murine α-1,3,-galactosyl transferase (GalT) gene, 2 μl of this mixture was used as a substrate for PCR amplification using the primers:

GALT-F2: CAC AGA CAG ATC TCT TCA GG [SEQ ID NO:11]

and

GALT-R1: ACT TTA GAC GGA TCC AGC AC [SEQ ID NO:12].

The PCR amplification was performed using HotStarTaq DNA polymerase according to the manufacturer's protocol (Qiagen). PCR amplification conditions involved an initial activation step at 95°C for 15 mins, followed by 35 amplification cycles of 94°C for 30 secs, 55°C for 30 secs and 72°C for 60 secs, with a final elongation step at 72°C for 4 mins. The PCR amplified region of GalT was column purified (PCR purification column, Qiagen) and then cloned into pCR2.1-TOPO according to the manufacturer's instructions (Invitrogen), to make plasmid TOPO.GALT.

(b) Test plasmid

Plasmid pCMV.GALT.BGI2.TLAG

Plasmid pCMV.GALT.BGI2.TLAG (illustrated in Annexure KCR2) contains an inverted repeat, or palindrome, of a region of the murine 3'UTR GalT gene that is interrupted by the insertion of the human β-globin intron 2 sequence therein. Plasmid pCMV.GALT.BGI2.TLAG was constructed in successive steps: (i) the GALT sequence from plasmid TOPO.GALT was sub-cloned in the sense orientation as a BglII-to-BamHI fragment into BglII-digested pCMV.BGI2 to make plasmid

E

pCMV.GALT.BGI2, and (ii) the GALT sequence from plasmid TOPO.GALT was sub-cloned in the antisense orientation as a BglII-to-BamHI fragment into BamHI-digested pCMV.GALT.BGI2 to make plasmid pCMV.GALT.BGI2.TLAG.

2. *Generation of transgenic mice*

Transgenic mice were generated through genetic modification of pronuclei of zygotes. After isolation from oviducts, zygotes were placed on an injection microscope and the transgene, in the form of a purified DNA solution, was injected into the most visible pronucleus (US patent number: 4,873,191).

Pseudo-pregnant female mice were generated, to act as "recipient mothers", by induction into a hormonal stage that mimics pregnancy. Injected zygotes were then either cultured overnight in order to assess their viability, or transferred immediately back into the oviduct of pseudo-pregnant recipients. Of 99 injected zygotes, 25 were transferred. Transgenic off-spring resulting from these injections are called "founders". To determine that the transgene has integrated into the mouse genome, off-spring are genotyped after weaning. Genotyping was carried out by PCR and/or by Southern blot analysis on genomic DNA purified from a tail biopsy.

Founders are then mated to begin establishing transgenic lines. Founders and their offspring were maintained as separate pedigrees, since each pedigree varies in transgene copy number and/or chromosomal location. Therefore, each transgenic mouse generated by pronuclear injection was the founder of a new strain. Where the founder was female, some pups from the first litter were analyzed for transgene transmission.

Transgenic mice were identified by Southern blots as follows. Annexure KCR5 is a Southern blot of GalT(hp) transgenic founders. DNA was extracted from tail tip samples of mice born from zygotes injected with a GalT hairpin construct (GalT(hp)) into the male pronucleus, as described. Samples were digested with BamHI and probed with the CMV promoter. Mice #74-026, #74-028, #74-034 were identified as transgenic (male, male, female, respectively); #74-028 was subsequently revealed to contain two unlinked integrations of the construct. Annexure KCR6 is a Southern blot of A-generation progeny of GalT(hp) transgenic founder #74-026. DNA was extracted from tail tip samples of A-generation progeny A013, A014 and A015, as shown. Samples were digested with BamHI and probed with the CMV promoter. A-

generation mice were bred from mating #74-026 with a C57Bl/6 female. Mice #74-A013 (male) and #74-A015 (male) were identified as transgenic (female). Annexure KCR7 is a Southern blot of A-generation progeny of GalT(hp) transgenic founder #74-028. DNA was extracted from tail tip samples of A-generation progeny A022-A029, A032 and A033, as shown. Samples were digested with BamHI and probed with the CMV promoter. A-generation mice were bred from mating #74-028 with a C57Bl/6 female. Mouse #74-A025 (male) was identified as transgenic, containing one of the segregated GalT(hp) insertions of founder #74-028.

3. Detection of co-suppression phenotype

The enzyme α-1,3,-galactosyl transferase (GalT) catalyzes the addition of galactosyl sugar residues to cell surface proteins in cells of all mammals except humans and other primates. The epitope enabled by the action of GalT is the predominant antigen responsible for the rejection of xenotransplants in humans. Cytological analyses of GalT expression levels in peripheral blood leukocytes (PBL) and splenocytes using FACS confirms the down regulation of the gene's activity.

To analyze cells from transgenic mice transformed with the GalT construct, FACS assays on peripheral blood leukocytes (PBL) and splenocytes are undertaken. White blood cells are the most convenient source of tissue for analysis and these were isolated from either PBL or splenocytes. To isolate PBL, mice are bled from an eye and 50 to 100 μl of blood collected into heparinized tubes. The red blood cells (RBCs) are lysed by treatment with NH_4Cl buffer (0.168M) to recover the PBLs. Annexure KCR8 is a FACScan analysis of peripheral blood lymphocytes from GalT(hp) transgenic mice. Transgenic mice and littermate controls were eye-bled into heparinised tubes (all manipulations were done on ice). Red blood cells were lysed and lymphocytes recovered by centrifugation and fixed in 4% paraformaldehyde in PBS. The cells were dual-labelled for Thy-1 and galactosyl residues with anti-Thy-1 MAb-FITC and lectin IB4-biotin, respectively. After washing, the cells were incubated with streptavidin-Cy5, washed and analysed by dual-channel analysis using a FACScan. Samples from A-generation mouse (as in previous figures) 026-A015 were markedly reduced in lectin binding, as shown, reflecting down-regulation of GalT.

E

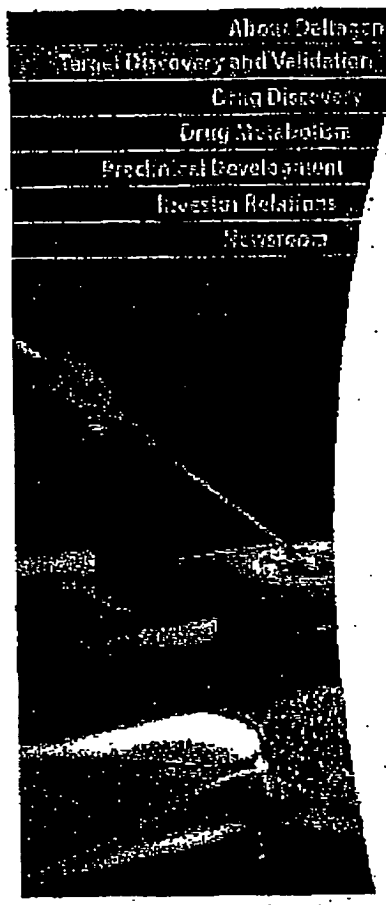
I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

Date Sep 26, 2002 By _____

Ken C Reed

Deltagen

Bringing drug discovery to life.



In Vivo Mammalian Technology

Deltagen has industrialized a revolutionary *in vivo* technology platform for the large-scale generation of functional information on genes related to key areas of drug discovery. Our new facilities give us the ability to rapidly create and characterize more than 1,000 mouse gene knockouts per year—exponentially more than was ever thought possible.

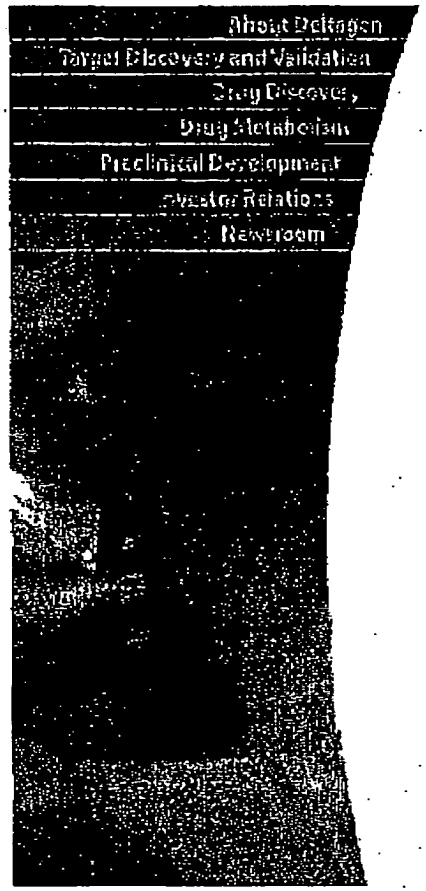
Using a preferred, targeted mutagenesis process, our scientists can delete—or "knock out"—predse genes in mouse models and then utilize an extensive phenotypic analysis program to determine the specific function and potential pharmaceutical relevance of these genes and the proteins they encode. The data are consolidated in DeltaBase™, Deltagen's premier *in vivo* mammalian gene function database. The proprietary technology platform has been validated through collaborations with some of the world's leading pharmaceutical companies, including Pfizer Inc., GlaxoSmithKline plc, and Merck & Co., Inc., as well as with internationally recognized research institutions such as Stanford University.

THE IDEAL MAMMALIAN MODEL

The mouse genome is more than 99 percent homologous to the human genome, making it the model of choice for drug target validation.

Deltagen

Bringing drug discovery to life.



Press Releases

Deltagen Announces Research Collaboration With Stanford University

REDWOOD CITY, Calif., Feb. 19 /PRNewswire-FirstCall/ -- Deltagen, Inc. (Nasdaq: DGEN) announced today that they have signed a target validation and research collaboration agreement with Stanford University.

Under the terms of the three-year collaboration, Stanford and Deltagen will mutually develop research projects for jointly selected genes under which Deltagen will provide Stanford non-exclusive access to knockout mice models using its proprietary high throughput technology and Stanford will evaluate and conduct research on such materials. Deltagen will have options to obtain exclusive licenses to commercially develop in any and all fields certain inventions developed by Stanford. Deltagen shall have rights to use, commercialize and sublicense results developed by Stanford under the research projects. No financial terms were disclosed.

"We have undertaken a comprehensive systems biology approach to unlocking the potential of the genome. In this endeavor we are generating several thousand genetic in vivo models we believe can greatly facilitate medical research. These models are of great value to academic researchers and we are eager to establish mutually beneficial collaborations with leading institutions such as Stanford. We look forward to combining Stanford's scientific excellence with Deltagen's talents in our search toward exciting new medical discoveries," said William Matthews, Ph.D., chief executive officer at Deltagen.

Deltagen is a biopharmaceutical company headquartered in Redwood City, California, and a world leader in the area of in vivo mammalian gene function information. Understanding the function, role and disease relevance of mammalian genes may facilitate the discovery and validation of drug targets and advance the development of new genomic-based medicines. Deltagen's principal

product, DeltaBase(TM), provides a database of in vivo derived, mammalian gene function information. In addition, the company is dedicated to determining the function of secreted proteins and is undertaking the discovery and development of biotechnology drug candidates internally or in collaboration with other parties. Deltagen currently has secreted protein agreements with Lilly and Hyseq, Inc. Current DeltaBase collaborators include Pfizer, Inc., GlaxoSmithKline plc, Merck & Co., Lexicon Genetics, Inc. and Vertex Pharmaceuticals, Inc.

This press release contains forward-looking statements that are subject to risks and uncertainties that could cause actual results to differ materially from those set forth in the forward-looking statements, including uncertainties related to our ability to identify successful drug targets, Stanford's ability to develop commercially relevant inventions, uncertainties related to product and drug development, Deltagen's dependence on proprietary technology, uncertainties related to third-party patents and other intellectual property, and other risks cited in the risk factors sections of the Deltagen's Annual Report on Form 10-K filed with the Securities and Exchange Commission and Deltagen's other securities filings with the Commission. These forward-looking statements speak only as of the date hereof. Deltagen disclaims any intent or obligation to update these forward-looking statements.

Deltagen

Bringing drug discovery to life.

Press Releases

Deltagen and Merck Enter Into DeltaBase License Agreement

REDWOOD CITY, Calif., Feb. 11 /PRNewswire-FirstCall/ -- Deltagen, Inc. (Nasdaq: DGEN) announced today that it has entered into a license agreement to provide Merck & Co., Inc. (NYSE: MRK) with access to Deltagen's proprietary DeltaBase (TM) product, a powerful resource tool for the understanding of in vivo mammalian gene function information.

Merck will have non-exclusive access to information related to 750 genes selected for their biological interest that have been functionally characterized and entered into DeltaBase. Merck will also have access to certain of the corresponding DeltaBase intellectual property rights. Financial terms were not disclosed.

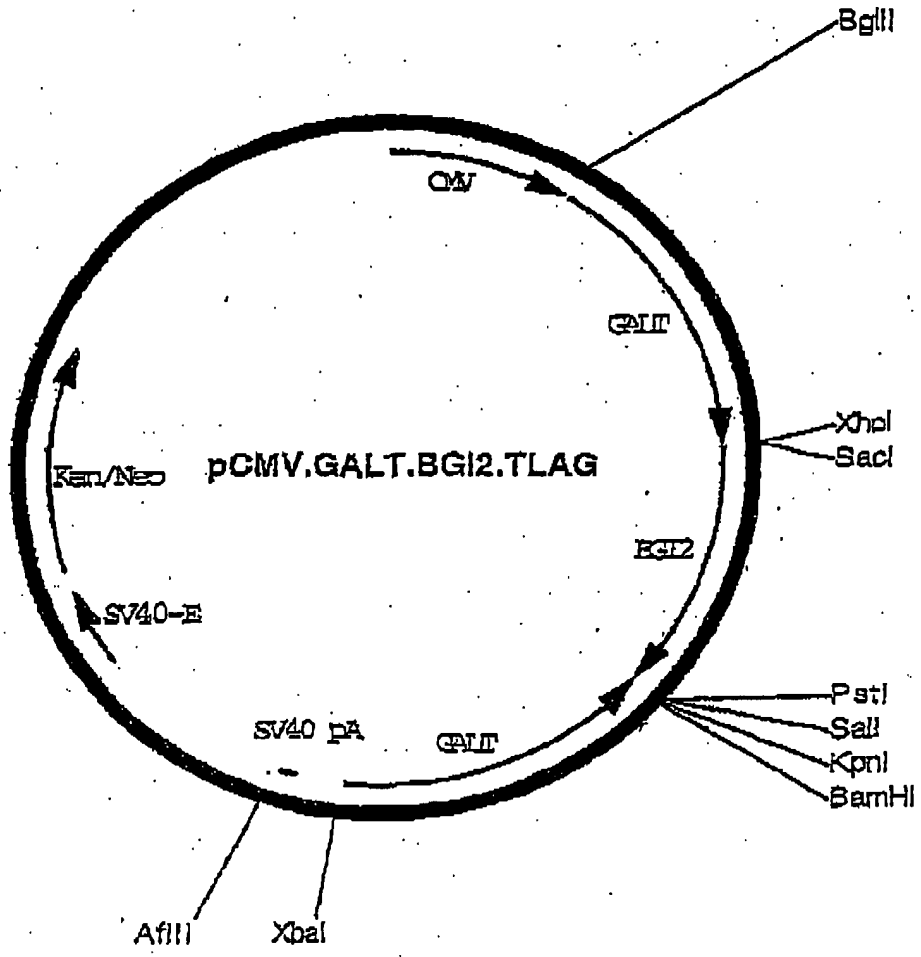
"We are delighted to extend our relationship with Merck, which has a worldwide reputation in pharmaceutical discovery and development, by adding them as a DeltaBase subscriber," said William Matthews, Ph.D., president and chief executive officer at Deltagen.

Through its proprietary product DeltaBase(TM), Deltagen provides pharmaceutical companies with critical information to better understand the in vivo function of mammalian genes, their relationship to other genes and the biochemical pathways for large segments of the genome. Each gene is the focus of an exhaustive investigation; more than 20,000 pieces of data from each gene are distilled into disease-relevant frameworks that include proprietary knockout mouse phenotypic, expression profile data and other proprietary target validation data. Information in DeltaBase is generated using Deltagen's large-scale mouse gene knockout technology and standardized phenotypic analysis protocols.

Deltagen is a genomic-based biotechnology company headquartered in Redwood City, California, that provides data to pharmaceutical and biotechnology companies on the function, role and disease relevance of mammalian genes. This

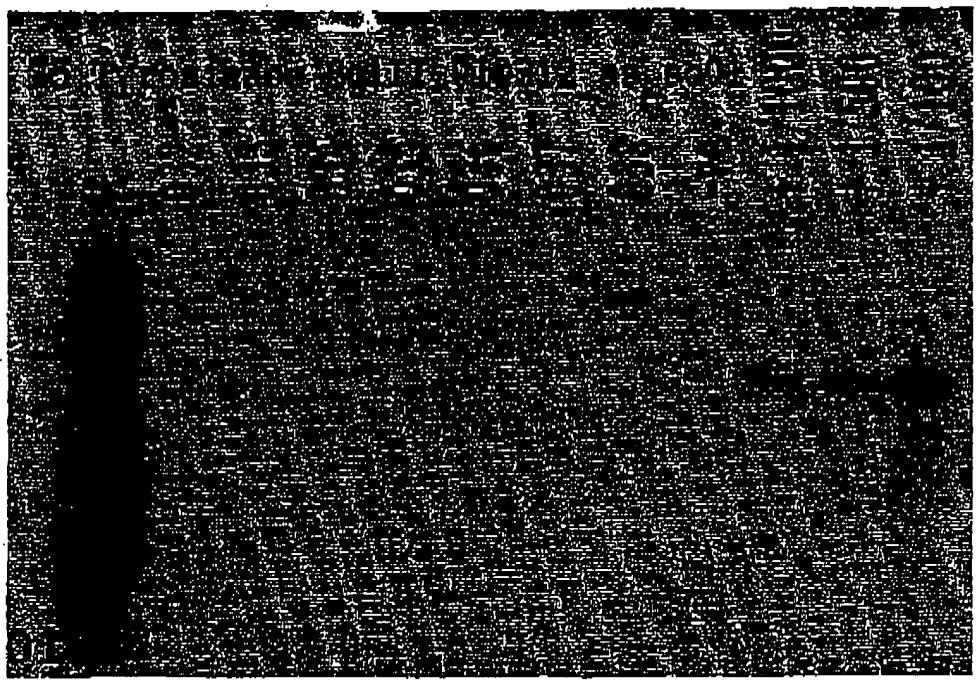
Information may facilitate the discovery and validation of drug targets to advance the development of new genomic-based medicines. Deltagen's principal product, DeltaBase, provides a database of in vivo derived, mammalian gene function information. In addition, the company is dedicated to determine the function of secreted proteins and is undertaking the discovery and development of biotechnology drug candidates internally or in collaboration with other parties. Current DeltaBase subscribers can be found on Deltagen's website, www.deltagen.com .

Except for the historical information contained herein, the matters set forth in this press release, including statements as to the role that Deltagen's DeltaBase product and gene function database information will play in third-party research programs and the extent to which genome-based research will assist researchers in their drug discovery efforts, are forward-looking statements within the meaning of the "safe harbor" provisions of the Private Securities Litigation Reform Act of 1995. These forward-looking statements are subject to risks and uncertainties that may cause actual results to differ materially from those set forth in the forward-looking statements, including the extent to which genomic databases are utilized in pharmaceutical research and development; the ability of Deltagen to provide products and services that meet market needs; the impact of competition and alternative technologies, processes and approaches; and other risks cited in the risk factors sections of Deltagen's Annual Report on Form 10-K filed with the Securities and Exchange Commission and Deltagen's other securities filings with the Commission. These forward-looking statements speak only as of the date hereof. Deltagen disclaims any intent or obligation to update these forward-looking statements.



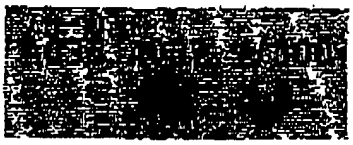
Annexure KC82

E



Annexure KCR3

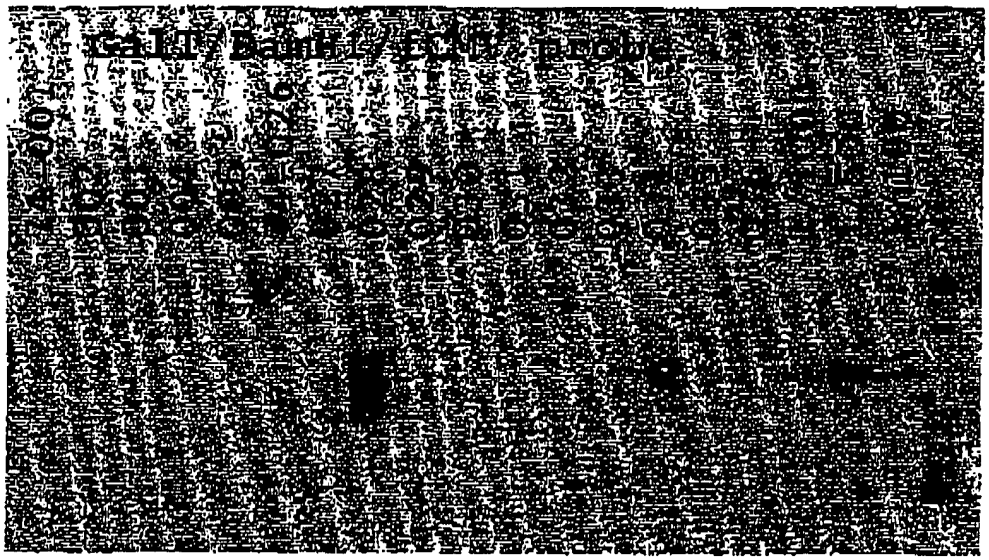
E



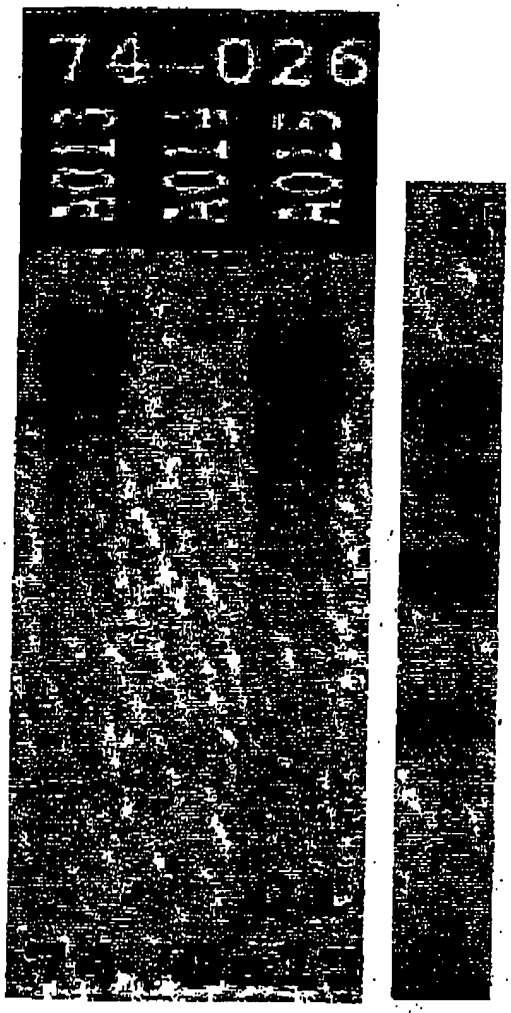
fCMV

Shiraz 3

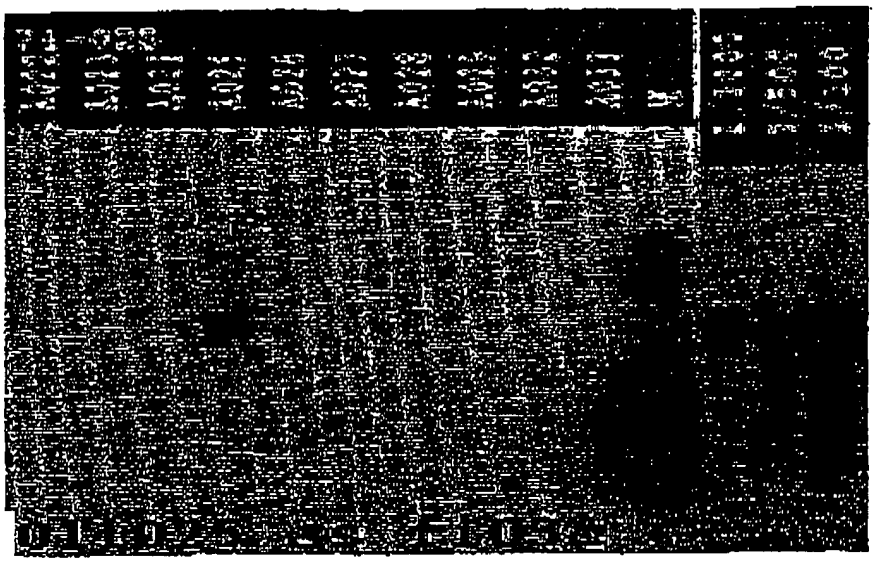
Annexure KCR4



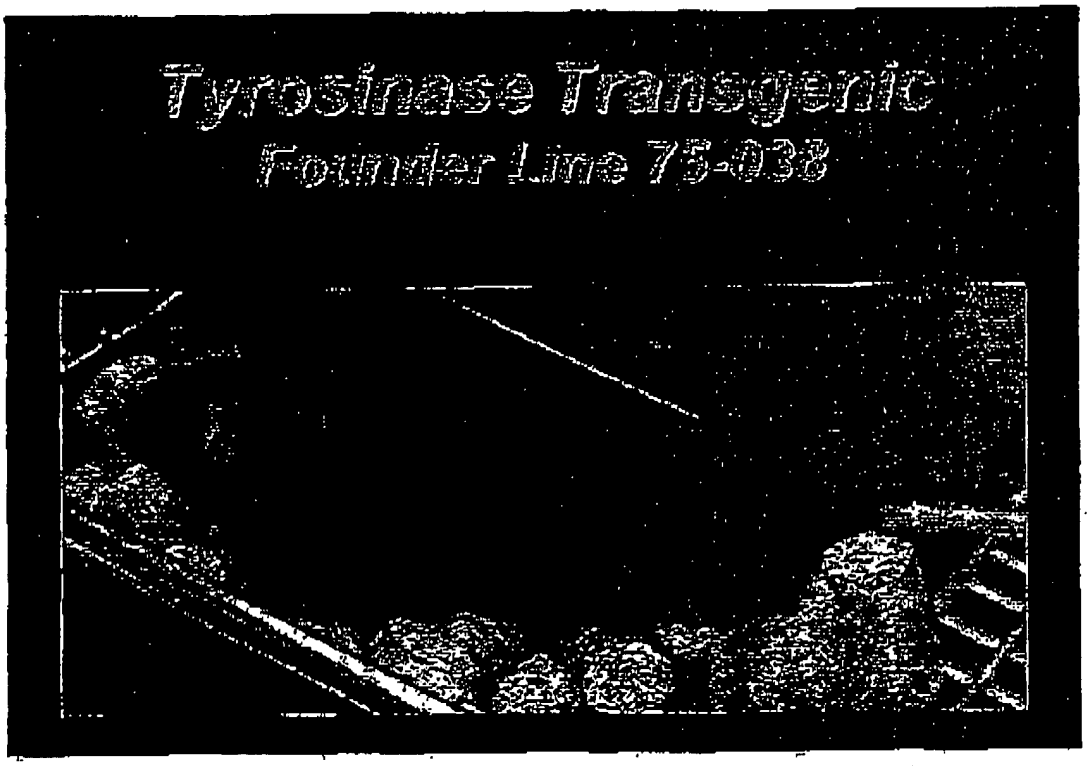
Annexure KCR5



Annexure KCR6

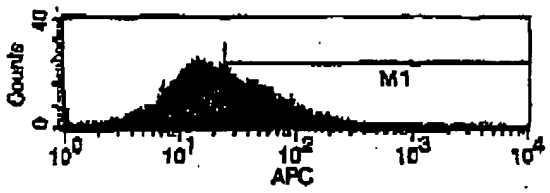


Annexure KCR7



Annexure KCR9

C57Bl/6

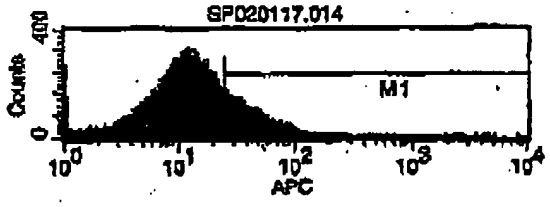


Mean = 22.2

File: SP020117.010

Marker	% Total	Geo Mean
All	60.10	22.79
M1	35.91	59.48

026-A015



Mean = 13.3

File: SP020117.014

Marker	% Total	Geo Mean
All	87.83	13.34
M1	21.09	49.36

Annexure KCR8